



Utilization of a probiotic-hemicellulases combination to prevent effects of *Salmonella* contamination of broilers[□]

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Introduction

Salmonella spp. remains a major cause of foodborne illness in humans worldwide. These may be carried asymptotically in the alimentary tract of live poultry, while deteriorating growth performances, and then spread via the slaughter process to raw, finished products. Among the strategies proposed to prevent intestinal colonization of poultry with *Salmonella* spp. is the use of enzymes such as hemicellulase (Remus, 2003) and microorganisms with probiotic potential (Pascual et al., 1999). Nevertheless, little is known about the effect of these products on performance parameters of the birds.

Objective

The objective of the study was to determine the effectiveness of a combination probiotic - hemicellulases by its relative incidence on gastrointestinal (GI) tract microbiota, correlated to the animal performances during the growing period of broilers infected or not with *Salmonella typhimurium*

Materials & Methods

Five groups of 54 male Cobb broilers were used in this experiment, conducted in a environmentally controlled room, with 6 birds/cage and 9 cages/treatment. One group consists of uninfected broilers fed with the basal diet (T-), and 4 groups are composed of *Salmonella* infected birds, with the basal diet for each group containing either 100 ppm of hemicellulase preparation (E), 10⁶ cfu/g diet of powdered probiotic (P), 100 ppm of hemicellulase preparation + 10⁶ cfu/g diet of powdered probiotic (PE), or no additive (T+). Probiotic strain was also administered to the broilers by spraying at the hatchery with a probiotic suspension of 10¹⁰ cfu/ml. Chicks were infected at 3 days of age with 10⁸ cfu of *Salmonella* per chick by oral gavage, in combination with a heat stress to intensify stress conditions for birds. Body weight and feed consumption are measured at 7, 14, 21 and 28 days of age. The relative incidence on GI tract microbiota of the different treatments was realized using Fluorescence *In Situ* Hybridization (FISH)(Harmsen *et al.*, 2002), with a set of 16SrRNA oligonucleotides probes targeted against almost all bacteria (Eub338), *Lactobacilli/Enterococci* (Lab158), *Clostridia* (Chis150/Clit135) and *E. faecalis/E. faecium* (Enf13/Enf2). The total number of cells was determined by staining with 4',6-diamidino-2-phenylindole (DAPI) for hybridization control. Feces samples were collected from 3 cages for each treatment.

Results & Discussion

Zootechnical performances

Effects of diet additives on performances are similar whatever the age period of broilers (Table 1). The infection pattern leads to a deterioration of the weight gain and the FCR by 36.8% and 38.9% respectively, in comparison with uninfected birds. The probiotic and the hemicellulases preparation significantly increase the performances of infected birds, with approximately the same body weight gain and FCR improvement for the 2 additives (+5.5% and +4.8% respectively). Moreover, combination of probiotic strain and hemicellulases significantly intensifies the growth performances improvement, increasing body weight gain by 17.1% and decreasing FCR by 7.2%, in comparison with non treated infected birds.

Table 1: Growth performances of broilers infected or not with *S.typhimurium* and fed a diet supplemented or not with hemicellulases and/or probiotic

Performances	diet					SEM
	T-	T+	E	P	PE	
Initial weight (g)	134.8	136.9	143.5	131.4	134.6	
Final weight (g)	1437	986	1060	1083	1145	
DM ing (g/j)	78.9 ^a	60.9 ^b	60.7 ^b	61.1 ^b	65.8 ^b	1.55
Daily weight gain (g/j)	64.2 ^a	41.8 ^b	45.7 ^c	46.7 ^c	49.7 ^d	1.12
FCR	1.17 ^a	1.40 ^b	1.31 ^c	1.31 ^c	1.28 ^c	0.017

FCR : feed conversion ration; DM: dry matter; SEM: standard error of means; T-, T+, E, P and PE :cf. Material & methods.

Means on the same line with different letters differ at the 5% level of significance

Microbial diversity analysis

Enumeration of genera *Clostridia*, *Enterococci/Lactobacillus*, *Bifidobacteria* and *E. faecalis/E. faecium* bacteria in feces samples shows significant differences between the treatments (Fig. 1).

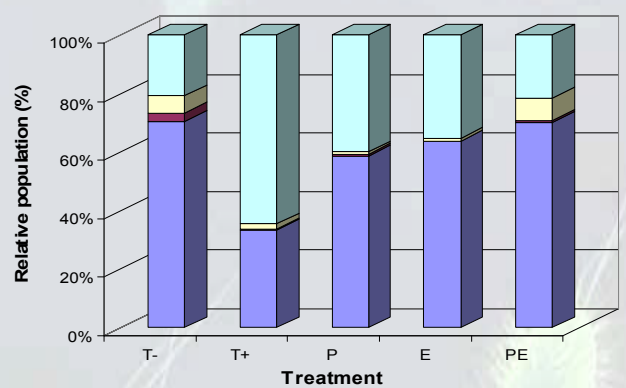


Figure 1 : Bacteria in feces of 14-days-old broiler hybridizing with oligonucleotides probes.

Compare to uninfected birds (T-), *Salmonella* infection (T+) increases the relative proportion of the *Enterococci/Lactobacillus* genus. In contrast, the *Bifidobacteria* population decreases. The reports between *Enterococci/Lactobacillus* and *Bifidobacteria* population are 0.37 and 1.9 for infected and uninfected broilers, respectively. The probiotic (P) and enzyme (E) treatment have approximately the same effect, the reports between *Enterococci/Lactobacillus* and *Bifidobacteria* are 0.81 and 0.57. Likewise, for *Clostridia* and *E. faecium/E. faecalis*, the relative proportions are similar of infected broilers. Combination of probiotic/enzyme (PE) improves the report *Enterococci/Lactobacillus* - *Bifidobacteria* (0.42) which is close up of uninfected broilers (0.37). Furthermore, the relative proportions of *Clostridia* and *E. faecium/E. faecalis* are similar to the healthy broilers.

Conclusions

Our data show that the probiotic-hemicellulases combination reduced the salmonellosis effects on broilers. Future research will involve the effectiveness of this combination against *Salmonella* cecal colonization.

References

- Harmsen H.J.M., Raangs G.C., He T., Degener J.E., Welling G.W., 2002. *Appl. Environ. Microbiol.*, **68**: 2982-2990.
Pascual M., Hugas M., Badiola J.L., Monfort J.M., Garriga M., 1999. *Appl. Environ. Microbiol.*, **65**: 4981-4986.
Remus J., 2003. *Feedstuffs*, **75**: 14-16.